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ALTERATIONS IN TISSUE METABOLISM (THE LUNG) WITH INJURY AND SHO--ETC(U)

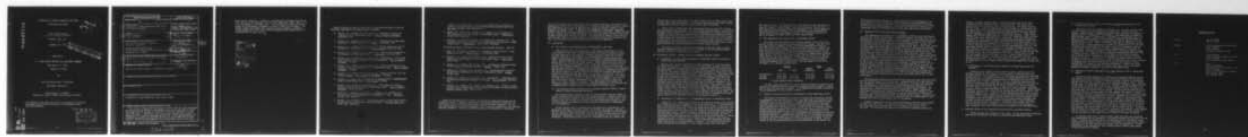
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ALTERATIONS IN TISSUE METABOLISM (THE LUNG)
WITH INJURY AND SHOCK

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Annual Summary Report

ARTHUR E. BAUE, M.D.

September 30, 1976

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The results indicate that the extent to which tissue ATP levels decreased during shock may be related to the metabolic activity of the organ or tissue. Associated with the decrease of tissue ATP levels was a decrease in tissue cyclic AMP levels. The results also indicate that the ability of tissue to regenerate ATP is markedly decreased during shock. Basal gluconeogenesis was unaltered during shock and steroids were found to be as effective in stimulating or inhibiting gluconeogenesis during hemorrhagic shock as they were		

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under control conditions. Evidence is presented which indicates that ATP can cross the intact cell membrane. During severe hemorrhagic shock, there is insulin resistance at the tissue level. This resistance to insulin on glucose uptake can be overcome by treatment of animals in shock with ATP-MgCl₂. Our results support the concept that inadequate shuttle enzymes is related to lactate production in the lung and that increased shuttle enzyme activity is associated with alloxan injury.

MgCl₂

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Recent progress can best be summarized by citing the publications from our laboratory supported by the previous years contract.

- a. Chaudry, I.H., Sayeed, M.M. and Baue, A.E. "Reversal of insulin resistance by ATP in hemorrhagic shock." Can. J. Physiol. Pharmacol. 53:859-865, 1975.
- b. Chaudry, I.H., Sayeed, M.M. and Baue, A.E. "Alterations in high-energy phosphates in hemorrhagic shock." Surgery 79:666-668, 1976.
- c. Chaudry, I.H., Sayeed, M.M. and Baue, A.E. "Energy factors and insulin resistance in hemorrhagic shock." Microcirculation 2:169-170, 1976.
- d. Chaudry, I.H., Sayeed, M.M., and Baue, A.E. "Failure of nicotinamide in the treatment of hemorrhagic shock." J. Surg. Res. 21:27-32, 1976.
- e. Chaudry, I.H., Sayeed, M.M., and Baue, A.E. "Differences in the altered energy metabolism of hemorrhagic shock and hypoxemia." Can. J. Physiol. Pharmacol. In press.
- f. Chaudry, I.H., Sayeed, M.M., and Baue, A.E. "Insulin resistance and its reversal by in vivo infusion of ATP in hemorrhagic shock." Can. J. Physiol. Pharmacol. In press.
- g. Chaudry, I.H., Sayeed, M.M., and Baue, A.E. "Uptake of ATP by liver and kidney in vitro." Can. J. Physiol. Pharmacol. In press.
- h. Chaudry, I.H., Baue, A.E. "Alterations in adenosine 3'-5'-monophosphate levels in hemorrhagic shock." Surg. Forum. In press.
- i. Chaudry, I.H., Sayeed, M.M. and Baue, A.E. "The effect of insulin on glucose uptake by soleus muscle in hemorrhagic shock." Can. J. Physiol. Pharmacol. 53:67-73, 1975.
- j. Krumholz, M., Sayeed, M.M., Romano, R., Chaudry, I.H., and Baue, A.E. "Low levels of α -glycerophosphate dehydrogenases in lung." Fed. Proc. 35:1509, 1976 (Abstract)
- k. Chaudry, I.H., and Baue, A.E. "Relationship within tissue organ function and high energy phosphates in hemorrhagic shock." Can. Fed. Biol. Soc. 19:68, 1976. (Abstract)

1. Chaudry, I.H., Adzick, N.S., Planer, G.J., Sayeed, M.M., and Baue, A.E. "Effect of glucocorticoids on gluconeogenesis during hemorrhagic shock." Fed. Proc. 35:2383, 1976. (Abstract)
- m. Sayeed, M.M., Perry, G.I., Romano, R.A., Chaudry, I.H., and Baue, A.E. "ATP regeneration by liver slices in hemorrhagic shock." Physiologist 18:379, 1975. (Abstract)
- n. Chaudry, I.H., Zweig, S., Planer, G.J., Sayeed, M.M. and Baue, A.E. "Relationship between nicotinamide adenine dinucleotide and survival in hemorrhagic shock." Physiologist 18:165, 1975. (Abstract)

The following papers which were in press have now been published. They are:

1. Baue, A.E. "Multiple, progressive or sequential systems failure - a syndrome of the 70's." Arch. of Surg. 110:779-781, 1975.
2. Chaudry, I.H., Sayeed, M.M. and Baue, A.E. "Reversal of insulin resistance by in vivo infusion of ATP in experimental shock." Surg. Forum. 26:44, 1975.
3. Chaudry, I.H., Sayeed, M.M. and Baue, A.E. "Evidence for enhanced uptake of ATP by muscle of animals in shock." Surgery 77:883, 1975.
4. Sayeed, M.M., Chaudry, I.H., and Baue, A.E. "Sodium-potassium transport and adenosine nucleotides in the lung in hemorrhagic shock." Surgery 77:395, 1975.
5. Sayeed, M.M., Senior, R.M., Chaudry, I.H., and Baue, A.E. "Characteristics of sodium and potassium transport in the lung." Am. J. Physiol. 229:1073, 1975.
6. Chaudry, I.H., Adzick, N.S., Sayeed, M.M. and Baue, A.E. "Effect of hydrocortisone and dexamethasone on xylose uptake by isolated rat soleus muscle." Biochem. Biophys. Acta 399:85, 1975.

Copies of the manuscripts 'In Press' or reprints of these publications are enclosed for review. A number of other papers are being prepared for submission for publication, but are not cited now because they have not been completed. Also we have participated in a number of programs in which the work supported by this contract has been presented. These include presentations by the responsible investigator

at the post-graduate courses at the Minneapolis Surgical Society, participation and presentation of our work in the Symposium on Trauma at Wayne State University, Detroit, and presentation of our work at the American College of Surgeon's Meeting in San Francisco in October, 1975. In addition, the responsible investigator presented several lectures as visiting professor at the following institutions: Medical College of Ohio, Toledo, Ohio; Montefiore Hospital, New York; New Jersey College of Medicine; Columbia Presbyterian Hospital, New York; Long Island Jewish Hospital, New York and various other lectures on regional and local problems on shock and circulatory failure. The principle findings of the past year will now be summarized.

1) Lung Studies

a) "Low levels of α -glycerophosphate dehydrogenase in the lung."

Lactate production from carbohydrates under aerobic conditions is a distinct feature of the lung. Similar production of lactate by neoplastic cells has been explained on the basis of inadequate levels of cytoplasmic "shuttle enzymes" required in the oxidation of extramitochondrial DPNH, as this promotes lactate production through oxidation of DPNH by lactate dehydrogenase (LDH). We have measured LDH and the shuttle enzyme α -glycerophosphate dehydrogenase (GPDH) in the lung and liver tissues. These enzymes were also measured in the lung with alloxan-induced injury. Lungs and livers of controlled rats and lungs of alloxan-treated (IV, 300 mg per kg body weight) rats were homogenized and mitochondria free supernatant prepared by differential centrifugation. LDH and GPDH activities in supernatant were assayed by a fluorometric procedure. The results (mean \pm S.E. values of enzyme activities in micromoles DPNH per mg. protein per minute) for LDH were 261.1 ± 11.9 , 72.6 ± 5.3 and 132 ± 14.7 for control liver, control lung and alloxan treated lung respectively. The values for GPDH were 269.2 ± 6.7 , $5.6 \pm .7$ and 24.8 ± 4.2 for control liver, control lung and alloxan treated lung respectively. Whereas the GPDH to LDH ratio was 1:1 in liver, it was 1:14 in lung. These data support the concept that inadequate shuttle enzymes is related to lactate production in the lung and that increased shuttle enzyme activity is associated with alloxan injury.

b) "The salutary effects of positive end expiratory pressure (PEEP), in experimentally induced pseudomonas pneumonia."

The cardiopulmonary response to positive end expiratory pressure (PEEP) in normal and hypovolemic animals has been studied in detail. However, controlled data on the effects of PEEP in the presence of pneumonia are unavailable. The present study was carried out to examine the cardiorespiratory effects of PEEP in canine pneumonia induced by the intra-tracheal inoculation of pseudomonas (4×10^9 organisms).

Sixteen mongrel dogs were anesthetized (pentobarbital, pancuronium), incubated and ventilated (16 cc/kg x 10/min) for 24 hours with 50% O₂ - 50% N₂O. One half of the animals were maintained with zero end expiratory pressure (ZEEP) and the remainder had 10 cmH₂O PEEP. Half of the dogs in each group were challenged with pseudomonas, and the following were measured (0,1,2,4,8,12,16 and 24 hours): blood pressure (BP), pulse (P), respiratory rate (R), dynamic com-

pliance (C_p), minute ventilation (V_E), tidal volume (V_T), pH, PaO_2 , $PaCO_2$, PvO_2 , cardiac output (CO), venous admixture (Q_s/Q_t), oxygen consumption (VO_2), pulmonary vascular resistance (PVR), hematocrit (HCT), and urine output.

Three of four infected ZEEP dogs died before 24 hours, while all infected PEEP and control dogs survived. The infected ZEEP dogs developed a significantly elevated cardiac index as early as 4 hours, accompanied by a highly significant increase in venous admixture; oxygenation and compliance deteriorated profoundly. Infected ZEEP dogs also showed signs of early capillary leak with a 50% increase in mean hematocrit, despite the infusion of twice the fluid load (13.3 cc/kg/hr) of the control group to maintain a pulmonary capillary wedge pressure of 9 mm Hg. Cardiovascular and respiratory function in the infected PEEP group was not markedly different than the non-infected PEEP or ZEEP control animals.

The results indicate a conspicuous advantage of PEEP over ZEEP in experimentally pseudomonas pneumonia, not only in terms of improved cardiopulmonary function, but also in terms of early survival.

2) Circulatory failure -- energy levels and membrane mediated effects.

a) "Alterations in high-energy phosphates in hemorrhagic shock as related to tissue and organ function.

Previous work from our laboratory has shown depletion of liver and kidney ATP levels during shock. In contrast to liver and kidney, skeletal muscle did not show a decrease in high-energy phosphate compounds unless the animals were in severe shock. Since the muscle used in the previous study was a resting muscle, it was, therefore, necessary to investigate the effect of hemorrhagic shock on a working muscle such as the diaphragm. To investigate this, hemorrhagic shock in rats were produced by cannulating the subclavian arteries and bleeding the animals to a mean arterial pressure of 40 mm Hg. This pressure was then maintained for one hour (early shock) or two hours (late shock). Analysis of the diaphragm (working muscle) showed that there was a significant decrease in ATP, ADP and Creatine phosphate levels beginning with early shock, whereas in soleus muscle these changes were not observed until late shock. Moreover, liver and kidney showed greater decreases in ATP levels than the diaphragm during various stages of shock. Thus in a working organ or tissue, ATP and Creatine phosphate levels decrease much earlier than in a resting skeletal muscle during shock. The results also indicate that the extent to which ATP levels decrease during shock may be related to the metabolic activity of the organ or tissue.

b) "Alterations in adenosine 3'-5'- monophosphate levels in hemorrhagic shock."

Adenosine 3'-5'- monophosphate (cAMP) is considered to regulate cell function and to mediate physiologic responses of cells to many stimuli. If the effectiveness of the cAMP system is decreased, this could result in loss of control of certain vital cellular activities. Since the formation of cAMP is through ATP, and since our previous work has shown that ATP levels were significantly reduced during hemorrhagic shock, it seems reasonable to expect that the cAMP system might also be altered during shock. To determine this, Albino Holtzman rats were cannulated, bled, and maintained at a pressure of 40 mm Hg for two hours. Following sacrifice, small pieces of liver, kidney, muscle, and brain were quickly removed and frozen in liquid nitrogen. Tissues were then homogenized and cAMP levels measured by the radioimmunoassay procedure. The results indicate that there are significant decreases in liver, kidney, muscle, and brain cyclic

AMP levels in shock. We have previously shown that with the same period of shock ATP levels in liver, kidney, and muscle decreased. The decreases in CAMP levels follow the same trend as the decreases in ATP levels suggesting that these events are related. The precise mechanism for decreased CAMP levels is not known at the present, however, it is possible that this is due to decreased ATP levels within the cells during shock.

c) "ATP regeneration by liver slices in hemorrhagic shock."

The purpose of this study was to determine whether liver slices from animals in shock were capable of regenerating ATP in vitro. Rats were bled to a mean arterial pressure of 40 mm Hg and maintained at this pressure until 30% of the shed blood was returned (1½ hours of shock). Animals were then sacrificed with or without reinfusion of the remaining shed blood and liver slices were prepared. Tissue ATP content was measured prior to and after chilling (0.5C for 90 min) of liver slices in a Krebs-Ringer phosphate (KRB) medium. The slices were then rewarmed (37C for 60 min) in a KRB and tissue ATP contents were determined. ATP content of chilled and rewarmed liver slices from unbled control animals were measured with and without 1.0 mM dinitrophenol (DNP) in KRB medium. The net change in liver slice ATP (mean \pm S.E. μ moles/g protein) were:

	<u>Control</u>		<u>Shock</u>	
	-DNP	+DNP	Before reinfusion	After reinfusion
Preincubation	11.9 \pm 0.6	*	3.1 \pm 0.8	4.2 \pm 0.7
Chilled	6.5 \pm 0.4	4.8 \pm 0.3	1.9 \pm 0.4	3.3 \pm 0.5
Rewarmed	11.2 \pm 0.6	3.1 \pm 0.4	4.0 \pm 0.6	5.7 \pm 0.8

* No DNP present before incubation

The above data shows that liver slices from both control and shock groups were able to regenerate ATP on rewarming. However, the net increase in tissue ATP in shock was about 50% of control. This approach now provides a method for further assessment of tissue energy turnover.

d) "Effect of glucocorticoids on gluconeogenesis during hemorrhagic shock."

This study was undertaken to determine if there was evidence of impaired gluconeogenesis during shock and to test the effectiveness of steroids on gluconeogenesis during such conditions. Holtzman rats were cannulated and bled to a mean arterial pressure of 40 mm Hg which was maintained for two hours. Control animals were treated in exactly the same manner but were not bled. After decapitation of the animals, liver slices (0.2 to 0.4 mm thick, average weight 30 mg) were prepared and incubated for three hours at 37C in Krebs-bicarbonate medium containing no added substrate or 10 mM alanine with or without hydrocortisone. Glucose and urea production in the medium was measured following incubation. Hydrocortisone addition at 10^{-7} M increased the quantity of glucose and urea appearing in the medium by 16% in the presence of control slices as well as in the presence of slices from animals in shock. The addition of 10^{-4} M hydrocortisone inhibited gluconeogenesis by 30% whereas with

10^{-2} M hydrocortisone there was a complete inhibition of gluconeogenesis with both groups of slices. Similar results were obtained using dexamethasone or hydrocortisone-21 sodium succinate. Thus, basal gluconeogenesis was unaltered during shock and steroids were as effective in stimulating or inhibiting gluconeogenesis during hemorrhagic shock as they were under control conditions.

e) "Uptake of ATP by liver and kidney in vitro."

Cell membranes are believed to be impermeable to ATP but many experimental observations indicate the contrary. A number of studies have shown that ATP is released from active skeletal muscle under conditions in which potassium was not. More recently, it has been shown that ATP is released from motor nerve terminal on indirect stimulation of mammalian nerve-muscle preparation. We have previously shown that ATP can enter intact skeletal muscle cells. From these studies, it would appear that the release and uptake of ATP is a physiological process. This being the case the process should also be present in tissues such as liver and kidney. In order to study this, rats were decapitated following which liver and kidney were quickly removed and tissues divided into small blocks (about 5 mm^3). Slices from liver and kidney (0.3 to 0.5 mm thick, average weight 40 mg) were then prepared from the tissue blocks with razor blades within 5 to 8 minutes after excision. Two to five slices from each organ were incubated for 1 hour at 37°C in 1.0 ml of Krebs-Henseliet bicarbonate buffer (pH 7.4) containing 10 mM glucose, 5 mM MgCl_2 and one of the following: 5 mM $[8-^{14}\text{C}]$ ATP (0.45 $\mu\text{C}/\mu\text{mole}$); 5 mM $[8-^{14}\text{C}]$ ADP (0.25 $\mu\text{C}/\mu\text{mole}$); 5 mM $[8-^{14}\text{C}]$ AMP (0.25 $\mu\text{C}/\mu\text{mole}$) or 5 mM $[8-^{14}\text{C}]$ adenosine (2 $\mu\text{C}/\mu\text{mole}$) under an atmosphere of 95% oxygen, 5% CO_2 in each case. At the end of the incubation period, slices were removed, rinsed quickly in ice-cold water, blotted on a dampened filter paper and frozen between aluminum blocks chilled in dry ice. The slices were then homogenized in 1.0 ml of a solution containing trichloroacetic acid (10%) and HCl (0.1 N) and centrifuged.

The supernatant solution was extracted four times with water-saturated ether and then neutralized with 1.0 M Tris base. Samples (50 μl) of tissue extract and incubation medium were applied to a Whatman No. 3 mm paper and subjected to electrophoresis. Following electrophoretic separation, radioactivity in the individual nucleotide spots was counted in a scintillation counter. The concentration of adenine and hypoxanthine nucleotides in medium ($\mu\text{moles/ml}$) and tissues ($\mu\text{moles/g}$) were calculated from the radioactivity observed in each fraction. A nucleotide was considered to have an intracellular distribution when the total tissue content exceeded the extracellular content. Extracellular concentrations were calculated on the assumption that the concentration in the extracellular water was the same as that of the medium.

Extensive degradation of the added nucleotide was observed in the presence of both liver and kidney. The concentrations of ^{14}C -ATP and ADP found in the liver and kidney indicated that these compounds were present within the cells. If the medium ATP levels at the end of the incubation period were

plotted, it became apparent that by maintaining higher medium ATP levels intracellular ATP increased in both liver and kidney. The relationship between medium ATP levels and intracellular ATP, i.e., ATP uptake, however, was not linear. There was approximately 100% increase in intracellular ATP when medium ATP levels were maintained at levels ranging from 0.18 μ moles/ml to 1.85 μ moles/ml. By further maintaining the medium ATP at levels extending to 3.5 μ moles/ml, intracellular ATP increased by 45% only. These results suggest that 14 C-ATP uptake activity was at a near saturation level when medium ATP levels were maintained above 1 μ moles/ml. Since the occurrence of saturation in uptake activity with increasing medium substrate concentration indicates the involvement of a membrane carrier in the uptake process, the results presented above suggest that ATP uptake in liver and kidney could be a carrier mediated process. The intracellular concentration of ATP in liver and kidney exceeds that in the medium at the lowest concentration tested (0.18 μ moles). Whether this suggests the existence of a low capacity active transport system for ATP is not known at present. These results indicate that liver and kidney cells are permeable to ATP and to a lesser extent to ADP and they suggest that the ATP uptake process in these organs could be a carrier mediated process. Previous work has shown that ATP can cross intact skeletal muscle cells. Thus, it would appear that ATP is capable of crossing cell membranes of liver, kidney, skeletal muscle and perhaps other tissue cells as well.

f. "Differences in the altered energy metabolism of hemorrhagic shock and hypoxemia."

Although tissue hypoxia has been thought to play a major role in the problems produced by circulatory failure and shock, it has been difficult to establish this and to separate it from other problems produced by decreased tissue perfusion. In studying alterations in energy metabolism with shock and how they may be corrected, it became apparent to determine what role tissue hypoxia may play in these alterations. We have, therefore, determined the effect of hypoxia per se, anoxia per se and hemorrhagic shock on the levels of pyridine and adenine nucleotides of various tissues. The results indicate that ATP levels in liver and kidney of animals in shock or animals subjected to seven minutes of anoxia decreased by 85% and 73% respectively. Under hypoxic conditions (arterial PO_2 18 mm Hg) the decrease was only 62% and 48% in liver and kidney respectively. Tissue NAD levels decreased and NADH levels increased during shock but were found to be essentially unaltered during experimental hypoxemia. Thus, shock produced greater alterations in adenine and pyridine nucleotides than did hypoxemia alone indicating that stagnant hypoxemia due to shock is more deleterious to energy metabolism than is severe hypoxemia with an otherwise normal circulation. The results also suggest that if an arterial PO_2 of 18 mm Hg represents the initial stages of tissue hypoxia, then tissue ATP levels are a more sensitive indicator of this than NAD levels.

3. Hormonal effects on cell membrane processes during circulatory failure.

Further studies were conducted in this area. We have published the detailed work now on the reversal of insulin resistance during hemorrhagic shock.

a) "Insulin resistance and its reversal by in vitro administration of ATP in hemorrhagic shock."

Hemorrhagic shock was produced by bleeding rats to a mean arterial pressure of 40 mm Hg which was maintained for two hours. Muscles from these animals ('shock muscles') showed resistance to the stimulation of glucose uptake by insulin. Addition of 1 mM ATP-MgCl₂ to the medium had no effect on basal glucose uptake in either group of muscles, but it permitted insulin to exert its stimulatory effect in shock muscle. An optimal insulin effect on glucose uptake in shock muscles incubated without ATP was observed at a concentration of 0.2 U/ml. When 1 mM ATP-MgCl₂ was added to the medium, optimal insulin effect in shock muscles was observed at a concentration of 0.007 U/ml. Increasing the concentration of ATP-MgCl₂ to 2.5 mM in the medium resulted in an optimal insulin effect at an insulin concentration of 0.001 U/ml in shock muscles. Following one hour of incubation in Krebs-bicarbonate medium, intracellular ATP contents of shock muscles were approximately 50% lower than in control muscles. Addition of 1 mM ATP-MgCl₂ to the incubation medium had no effect on the intracellular ATP contents of either group of muscles following incubation. However, 2.5 mM ATP-MgCl₂ elevated intracellular ATP contents of shock muscles but had no effect in control muscle. We have previously proposed that the insulin resistance in tissues from animals in shock could be due to a change in the membrane conformation. Whether the effect of ATP-MgCl₂ is due to reversal of cellular swelling or due to some other membrane or metabolic effect is not known at the present time.

b) "Reversal of insulin resistance by in vivo infusion of ATP in hemorrhagic shock."

Having found that addition of ATP-MgCl₂ to the medium containing muscles from animals in shock reversed the tissue insulin resistance, we then considered the possibility of infusing ATP-MgCl₂ to animals in shock to investigate whether this would also reverse the insulin resistance seen in shock. Following two hours of hypotension at 40 mm Hg, ATP-MgCl₂, ADP-MgCl₂, adenosine-MgCl₂ or GTP-MgCl₂ (25 μ moles each, 0.25 ml) was infused to animals followed by the return of the remaining shed blood. Fifteen minutes following the return of the shed blood, the animals were sacrificed. Muscles were then removed and the effect of insulin on glucose uptake in these muscles were studied. Infusion of ATP-MgCl₂, ADP-MgCl₂, adenosine-MgCl₂ or GTP-MgCl₂ to animals following shock had no effect on basal glucose uptake; however, treatment with ATP-MgCl₂ but not with the other nucleotides permitted insulin to exert its stimulatory effect on such muscles. An optimal insulin effect in ATP-MgCl₂ treated shock muscle occurred at an insulin concentration of 0.001 U/ml which is also the concentration required to produce optimal insulin effect in control muscles. Following one hour of incubation in Krebs-bicarbonate medium, intracellular contents of shock muscles were approximately 50% lower than in control muscles. Treatment with ATP-MgCl₂ following shock, however, resulted in ATP contents in such muscles similar to those in control muscles. The exact mechanism for the reversal of insulin resistance by ATP-MgCl₂ infusion in shock is not known at present.

It has been shown that in humans severe injury and shock are associated with hyperglycemia and an abnormality in glucose tolerance that persists after the injury. Although the present results indicate that insulin resistance during shock could be overcome by administration of ATP-MgCl₂, a strong caveat regarding hasty clinical application at the present stage is appropriate. Further experiments on the mechanism of this reversal and the possible side effects of such infusions are needed.

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